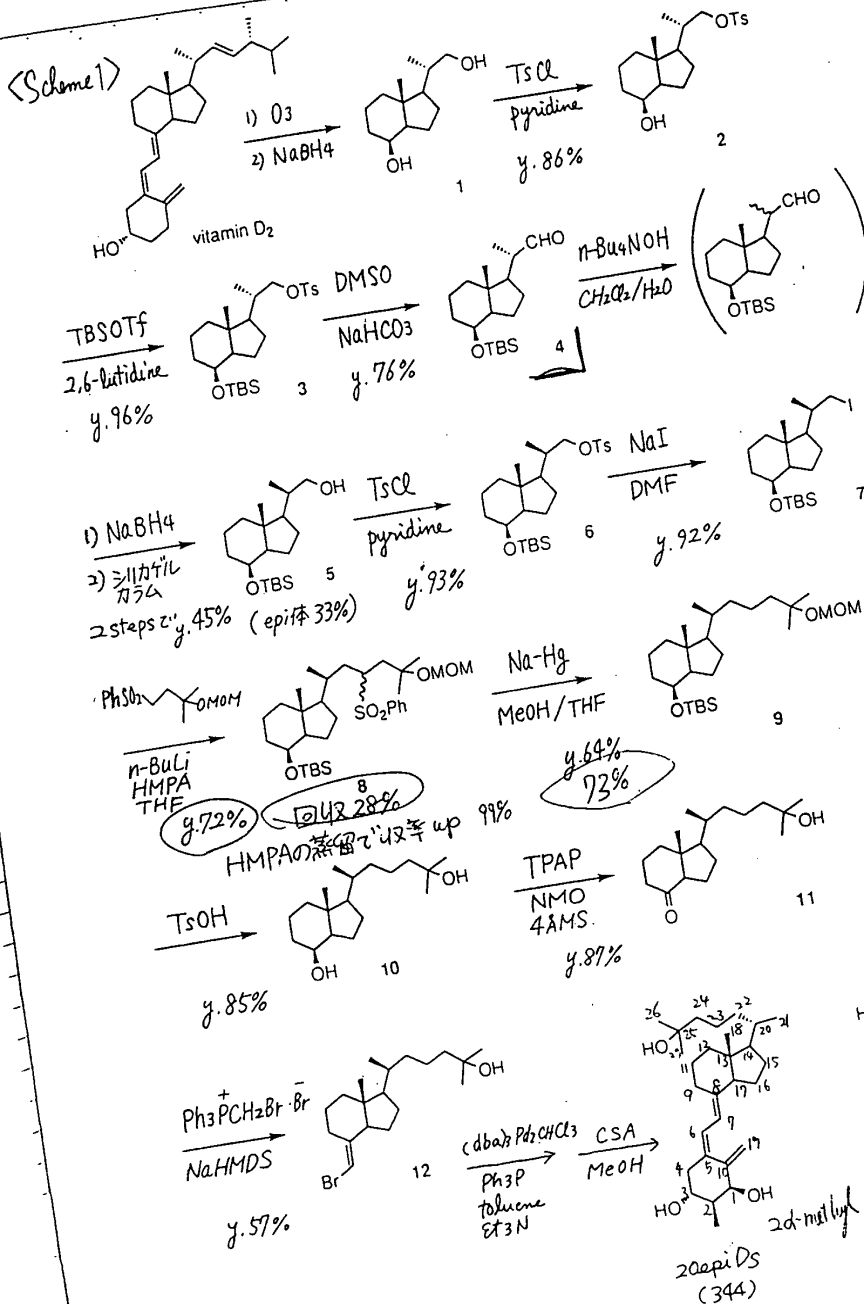




実験セミナー NO.3  
藤島利江.  
2-methyl-20 $\alpha$ epi 1 $\alpha$ ,25(OH) $_2$ VD $_3$ 誘導体の合成



# < Bovine Thymus VDR への結合実験 >

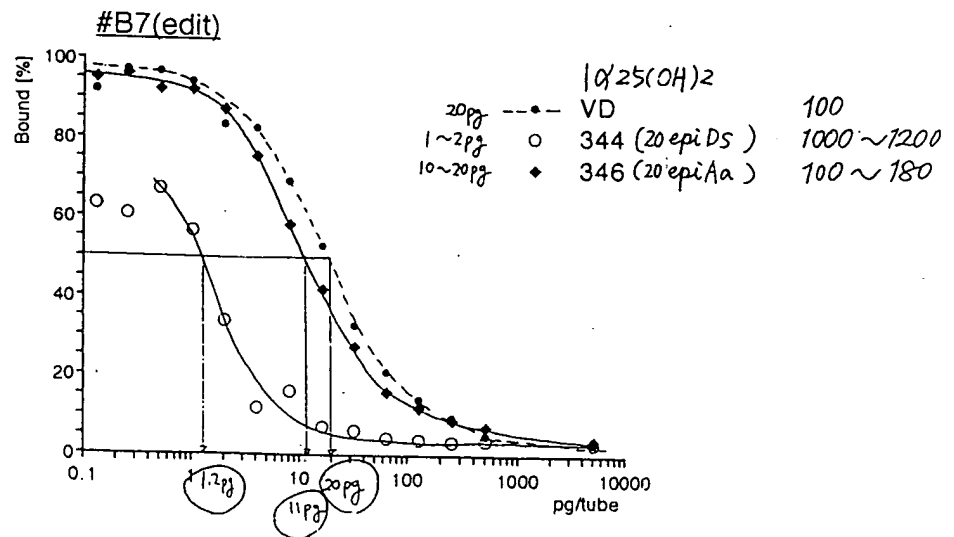
① 1) 酸カリ buffer {  $K_2HPO_4$  0.05M pH 7.4  
 $KH_2PO_4$  0.05M  
 $KCl$  0.3M  
 $DTT$  5mM

②  $1\alpha,25(OH)_2VD_3$  #344, #346 の  $\lambda_{max}$  の  $\epsilon = 18000\epsilon$  用いて濃度調整し希釈系列を作成する。

ウシ胸腺ビタミン D レセプターはヤマサ醤油株式会社より購入し (lot. 110431) 1 アンプル (約 25mg) を 0.05M リン酸 0.5M カリウムバッファー (pH 7.4) 55 ml に溶解した。ビタミン D 誘導体のエタノール溶液 50  $\mu$ l とレセプター溶液 500  $\mu$ l を室温で 1 時間ブレインキュベートした後、 $1\alpha,25(OH)_2[^3H]VD_3$  溶液 50  $\mu$ l を最終濃度 0.1nM となるように加えて 4℃ で一晩インキュベートした。結合と非結合の  $1\alpha,25(OH)_2[^3H]VD_3$  はデキストラン-コーテッド-チャコール処理して遠心分離し、上澄に液シンカクテル (ACS-II) を加えて放射活性をカウントした。

freeの drug が DCC にくっついて遠沈する。

ビタミン D 誘導体の活性は 50% 結合阻害する濃度を  $1\alpha,25(OH)_2VD_3$  を 100 としたときの比で表し評価した。



c.f. 20epi  $1\alpha,25(OH)_2VD_3$  の VDR への結合活性  
 • chicken intestine VDR 120  
 • bovine thymus VDR 500

7 → 8 (#323)

側鎖部 sulfone 980 mg (3 eq) in dry THF (1.5 ml) を Ar 雰囲気下、  
HMPA 1.5 ml (7 eq) を加え一様とした後、 $-78^{\circ}\text{C}$  に冷却した。  
n-BuLi (1.6 M in n-hexane) 2.3 ml (3 eq) を滴下し  $-78^{\circ}\text{C}$  で  
20 min かくはん後 3-ド体 7 525 mg (1.20 mmol) in dry THF  
(2 + 洗い込み 1 ml) を滴下。  $-78^{\circ}\text{C}$  で 1 hr かくはん後 反応液に  
sat  $\text{NH}_4\text{Cl}$  を加えて EA 抽出。有機層をあわせて brine で洗い、 $\text{MgSO}_4$  上  
脱水。30% エバポレート シリカゲルカラム (EA:n-hex=1:8) にて精製し  
無色 oil 503 mg (y. 72%) を得ると共に 1145 mg の原料を回収 (28%)。

8  $^1\text{H-NMR}$  ( $\text{CDCl}_3/\text{TMS}/400\text{MHz}$ )  $\delta$  -0.02 (3H, s) 0.00 (3H, s)  
0.66 (3H, d,  $J=6.4\text{Hz}$ ) 0.85 & 0.88 (3H, s) 1.23 & 1.27  
(3H, s) 2.32 (1H, dd,  $J=15.3\text{Hz}, 4.3\text{Hz}$ ) 3.26 (1H, m)  
3.30 (3H, s) 3.96 (1H, m) 4.57 (1H, d,  $J=7.3\text{Hz}$ ) 4.67  
(1H, d,  $J=7.3\text{Hz}$ ) 7.55 (2H, t,  $J=6.3\text{Hz}$ ) 7.63 (1H, t,  
 $J=6.3\text{Hz}$ ) 7.88 (2H, d,  $J=6.3\text{Hz}$ )

MS = 580 ( $\text{M}^+$ )

HRMS: calcd for  $\text{C}_{32}\text{H}_{56}\text{O}_5\text{SiS}$  = 580.3620  
found = 580.3618

8 → 9 (#310)

8 165 mg (0.28 mmol) を dry THF 3 ml, dry MeOH 3 ml と共に  
 $\text{Na}_2\text{HPO}_4$  3.0 g, 5% Na-Hg 9.8 g を加えて Ar 下 rt でかくはん  
overnight。反応液を ether で希釈し セライト 30% 有機層を  
brine で洗い、 $\text{MgSO}_4$  上脱水。30% エバポレート シリカゲル  
カラム (EA:n-hex=1:9) にて精製し 9 無色 oil 80 mg (y. 64%) を  
得ると共に原料 11 mg (7%) を回収。

9  $^1\text{H-NMR}$  ( $\text{CDCl}_3/\text{TMS}/400\text{MHz}$ )  $\delta$  -0.01 (3H, s) 0.01 (3H, s)  
0.81 (3H, d,  $J=6.7\text{Hz}$ ) 0.89 (9H, s) 0.91 (3H, s) 1.21  
(6H, s) 0.98-1.57, 1.64-1.94 (19H, m) 3.36 (3H, s) 3.99  
(1H, m) 4.70 (2H, s)

MS: 440 ( $\text{M}^+$ ), 425 ( $\text{M-Me}^+$ )

HRMS: calcd for  $\text{C}_{26}\text{H}_{52}\text{O}_3\text{Si}$  = 440.3688  
found = 440.3687

9 → 10 (#316)

ホジ体 9 80mg (0.18 mmol) を MeOH 3 ml に溶解し、TsOH·H<sub>2</sub>O 174mg (0.91 mmol) を加えて rt で攪拌 overnight. 反応液から MeOH とエバポレートし、シリカゲルカラム (EA:n-hex = 1:2) にて精製. 無色 oil 43mg (y. 85%) を得る.

10 <sup>1</sup>H-NMR (CDCl<sub>3</sub>/TMS/400MHz) δ 0.84 (3H, d, J=6.7Hz) 0.93 (3H, s) 1.21 (6H, s) 4.07 (1H, m)

MS: 264 (M-H<sub>2</sub>O)<sup>+</sup>, 246 (M-2H<sub>2</sub>O)<sup>+</sup>

HRMS: calcd for C<sub>18</sub>H<sub>32</sub>O : 264.2455 (M-H<sub>2</sub>O)  
found : 264.2453

10 → 11 (#326)

アルコール 10 117mg (0.41 mmol), dry CH<sub>2</sub>Cl<sub>2</sub> (10 ml), 4ÅMS 30mg を Ar 下 rt で 5 分間攪拌する. TPAP 84mg (0.24 mmol) を加えて 1 hr 20 min 後反応液を small pad of silica gel 上 3 かし. エバポレート. シリカゲルカラム (EA:n-hex = 1:1) にて精製. 100mg (y. 87%) を得る.

11 <sup>1</sup>H-NMR (CDCl<sub>3</sub>/TMS/400MHz) δ 0.64 (3H, s) 0.87 (3H, d, J=6.1Hz) 1.22 (6H, s) 2.45 (1H, dd, J=11.6Hz, 7.3Hz)

MS: 262 (M-H<sub>2</sub>O)<sup>+</sup>

HRMS: calcd for C<sub>18</sub>H<sub>30</sub>O (M-H<sub>2</sub>O) : 262.2298  
found : 262.2297

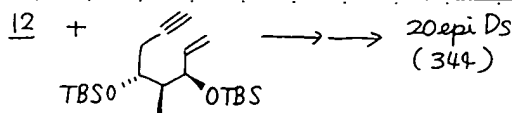
11 → 12 (#334)

(bromomethyl)triphenyl phosphonium bromide 389mg (5eq) in dry THF (1.5 ml) を Ar 下 -60°C に冷却し 1.0M NaHMDS 0.86ml (4.8eq) を加え -60°C で 1 hr 反応させた後. 11 50mg (0.18 mmol) in dry THF (1.5 ml) に transfer する. -60°C → 0°C → rt へと昇温し 1 hr 反応させた. 反応液に n-ヘキサンを加えセライト 3 かし 3 液をエバポレートしてシリカゲルカラム (EA:n-hex = 1:8 → 1:3) にて精製. 12 36mg (y. 56%) の淡黄 oil を得る.

12 <sup>1</sup>H-NMR (CDCl<sub>3</sub>/TMS/CDCl<sub>3</sub>) δ 0.56 (3H, s) 0.85 (3H, d, J=6.4Hz) 1.22 (6H, s) 2.88 (1H, m) 5.64 (1H, d, J=1.5Hz)

MS: 356 & 358 (M<sup>+</sup>), 338 & 340 (M-H<sub>2</sub>O)<sup>+</sup>

HRMS: calcd for C<sub>19</sub>H<sub>33</sub>O<sup>79</sup>Br : 356.1716  
found : 356.1715



12 17mg (0.048mmol) を toluene 0.3ml に溶かし Et<sub>3</sub>N 0.45ml を加える。Ar<sup>1</sup> (dba)<sub>3</sub>Pd<sub>2</sub>·CHCl<sub>3</sub> 1.9mg (0.03eq), Ph<sub>3</sub>P 2.5mg (0.3eq) を加え rt でかきはんしつ A 環部 13mg (0.034mmol) in toluene (150μl + 50μl) を加える。赤黒い溶液を rt で 10min かきはんすると黄色溶液となる。120°C の oil bath 上 2.5hr 反応させる。反応液をろか。ショートカラム (SiO<sub>2</sub>, EA=n-hex = 1:3) に付し黄色 oil を得る。(精製せず=次の反応へ。)

ホジ体と MeOH 1ml にとかし CSA 11mg (0.047mmol) を加えて Ar 下 rt で overnight かきはん。MeOH を溜去し水を加え EA 抽出。有機層を あつめ brine で洗う。MgSO<sub>4</sub> 上脱水ろかエバポレート。シリカゲルカラム (EA=n-hex=1:1) にて精製。無色結晶 9.3mg (y.63%) を得る。

#### <HPLCによる精製>

カラム: LiChrosorb RP-18 (7μm), 10×250, No. 301291

溶媒: Acetonitrile : 水 = 70 : 30

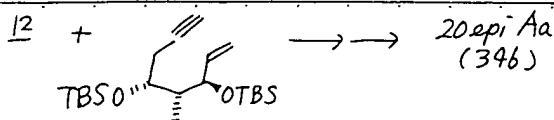
recycler をつて流速 7.0ml/min

$$\text{UV (EtOH)} : \begin{array}{l} \lambda_{\text{max}} 266\text{nm} \\ \lambda_{\text{min}} 226\text{nm} \end{array} \quad \frac{A_{226}}{A_{266}} = 0.57$$

<sup>1</sup>H-NMR (CDCl<sub>3</sub>-D<sub>2</sub>O / TMS / 400MHz) δ 0.53 (3H, s) 0.85 (3H, d, J=6.7Hz) 1.08 (3H, d, J=6.8Hz) 1.21 (6H, s) 1.12–2.04 (19H, m) 2.23 (1H, dd, J=7.9Hz, 13.4Hz) 2.67 (4.0Hz, 13.4Hz) 2.83 (1H, m) 3.83 (1H, ddd, J=7.9, 4.4, 4.0Hz) 4.29 (1H, d, J=3.3Hz) 5.01 (1H, d, J=1.8Hz) 5.28 (1H, m) 6.01 (1H, d, J=11.3Hz) 6.39 (1H, d, J=11.3Hz)

MS: 430 (M<sup>+</sup>), 412 (M-H<sub>2</sub>O)<sup>+</sup>, 394 (M-2H<sub>2</sub>O)<sup>+</sup>

HRMS: calcd for C<sub>28</sub>H<sub>46</sub>O<sub>3</sub> : 430.3447  
found : 430.3443



12 15 mg (0.042 mmol) を toluene 0.3 ml に溶かし Et<sub>3</sub>N 0.45 ml を加える (ArF) (dba)<sub>3</sub>Pd<sub>2</sub>·CHCl<sub>3</sub> 1.7 mg, Ph<sub>3</sub>P 2.5 mg を加え rt で 1 時間かくはん。A 環部 13 mg (0.034 mmol) in toluene (150 μl + 50 μl) を加え 10 min かくはん。120°C の oil bath 上 4 hr 反応させる。反応液をセライトでろ過し、ショートカラム (EA:n-hex = 1:3, SiO<sub>2</sub>) に付し。黄色油を得る。

ホコリ体と MeOH 1 ml にとかし CSA 11 mg (0.047 mmol) を加えて Ar 下 rt で overnight かくはん。MeOH を溜去し。ろくを加え EA 抽出。有機層を brine で洗う。MgSO<sub>4</sub> 上脱水ろ過。エバポレート。シリカゲルカラムにて (EA:n-hex = 1:1) 精製後 無色結晶 4.5 mg (y 31%) を得る。

<HPLCによる精製>

20<sup>epi</sup>-Ds と同様の条件

UV (EtOH): λ<sub>max</sub> 263 nm  
λ<sub>min</sub> 228 nm

$\frac{A_{\lambda_{min}}}{A_{\lambda_{max}}} = 0.55$

<sup>1</sup>H-NMR (CDCl<sub>3</sub>-D<sub>2</sub>O/TMS/400 MHz) δ 0.55 (3H, s), 0.85 (3H, d, J = 6.4 Hz), 1.15 (3H, d, J = 6.7 Hz), 1.21 (6H, s), 1.17–2.01 (19H, m), 2.42 (1H, dd, J = 13.9, 4.9 Hz), 2.52 (1H, d, J = 13.9 Hz), 2.82 (1H, dd, J = 11.9 Hz, 4.0 Hz), 3.99–4.04 (1H + 1H, m), 5.02 (1H, t, J = 1.8 Hz), 5.37 (1H, t, J = 1.8 Hz), 6.03 (1H, d, J = 11.3 Hz), 6.35 (1H, d, J = 11.3 Hz)

MS: 430 (M<sup>+</sup>), 412 (M-H<sub>2</sub>O)<sup>+</sup>, 394 (M-2H<sub>2</sub>O)<sup>+</sup>

HRMS: calcd for C<sub>28</sub>H<sub>46</sub>O<sub>3</sub> = 430.3447  
found 430.3441

## Experimental Seminar No. 3

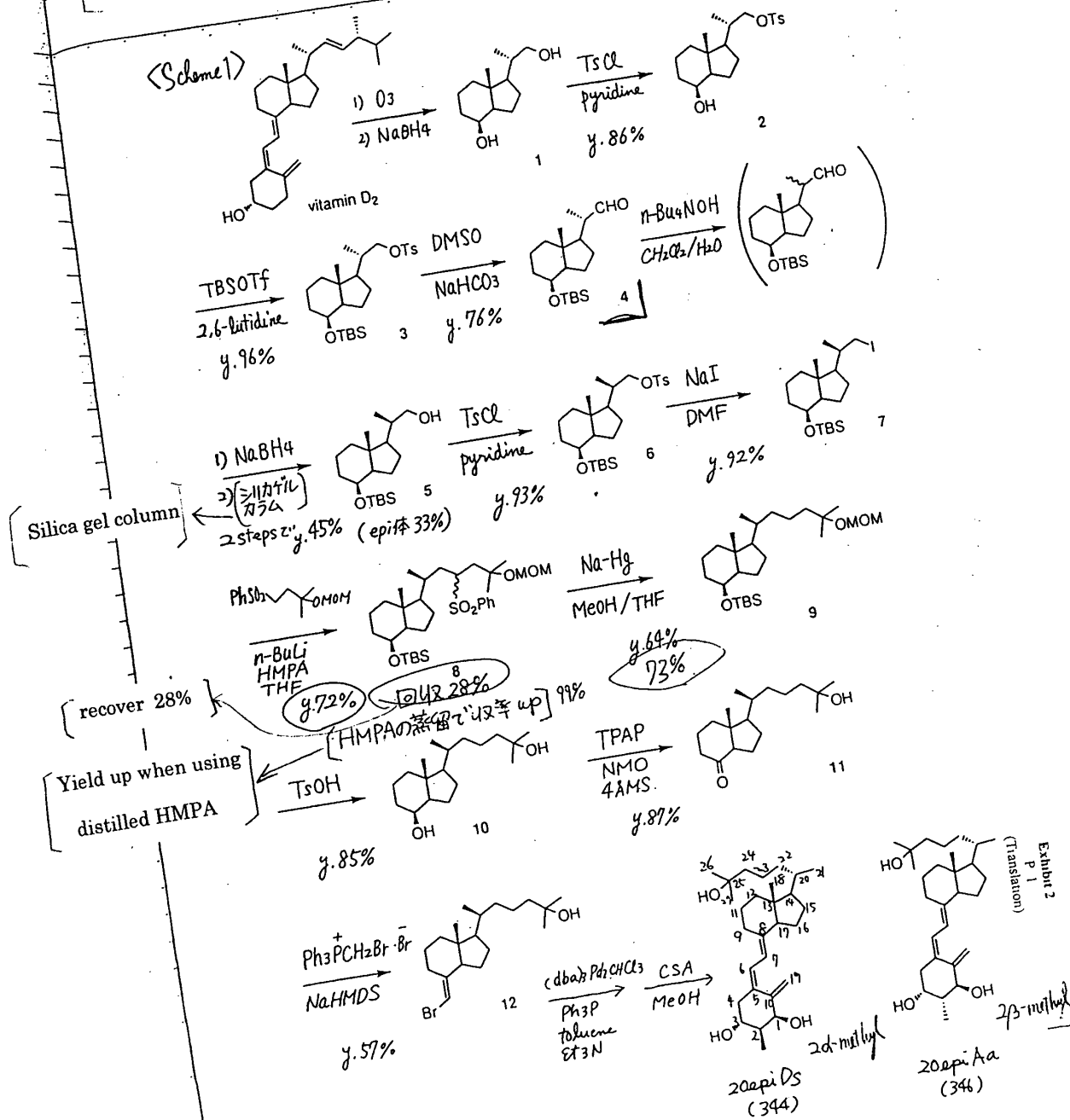
● Synthesis of 2-methyl-20epi 1 $\alpha$ ,25(OH) $_2$ VD $_3$  derivatives

実験セミナー No. 3

● 2-methyl-20epi 1 $\alpha$ ,25(OH) $_2$ VD $_3$ 誘導体の合成  
● 1 $\alpha$ ,25(OH) $_2$ VD $_3$ のA環部の合成法

藤島利江

&lt;Scheme 1&gt;



Make diluted solution series by concentration preparation of  $1\alpha,25(\text{OH})_2\text{VD}_3$ . #344, #346 according to  $\lambda_{\text{max}} \epsilon = 18000$ .

# <Bovine Thymus VDR の結合実験>

①リン酸カリ buffer

phosphate-potassium buffer

$\text{K}_2\text{HPO}_4$  0.05M  
 $\text{KH}_2\text{PO}_4$  0.05M  
 $\text{KCl}$  0.3M  
 $\text{DTT}$  5mM

pH 7.4

②  $1\alpha,25(\text{OH})_2\text{VD}_3$ , #344, #346 を  $\lambda_{\text{max}}$  の  $\epsilon = 18000$  を用いて濃度調整し希釈系列を作成する。

ウシ胸腺ビタミン D レセプターはヤマサ醤油株式会社より購入し (lot. 110431) 1 アンプル (約 25mg) を 0.05M リン酸 0.5M カリウムバッファー (pH 7.4) 55 ml に溶解した。ビタミン D 誘導体のエタノール溶液 50  $\mu\text{l}$  とレセプター溶液 500  $\mu\text{l}$  を室温で 1 時間ブレインキュベートした後、 $1\alpha,25-(\text{OH})_2[^3\text{H}]\text{VD}_3$  溶液 50  $\mu\text{l}$  を最終濃度 0.1nM となるように加えて 4°C で一晩インキュベートした。結合と非結合の  $1\alpha,25-(\text{OH})_2[^3\text{H}]\text{VD}_3$  はデキストラン-コーテッド-チャコール処理して遠心分離し、上澄に液シンカクテル (ACS-II) を加えて放射活性をカウントした。

free drug が DCC にくっついて遠沈する。

ビタミン D 誘導体の活性は 50% 結合阻害する濃度を  $1\alpha,25-(\text{OH})_2\text{VD}_3$  を 100 としたときの比で表し評価した。

The content (about 25 mg) of an ampule of a Bovine Thymus Vitamin D receptor (lot. 110431), which was purchased from YAMASA SYOYU KABUSHIKIGAISSYA, was dissolved in 55 ml of a 0.05 M phosphate 0.5 M potassium buffer (pH 7.4). After pre-incubation of 50  $\mu\text{l}$  of ethanol solution of Vitamin D derivative with 500  $\mu\text{l}$  of receptor solution for 1 hr at room temperature, 50  $\mu\text{l}$  of  $1\alpha,25-(\text{OH})_2[^3\text{H}]\text{VD}_3$  solution was added to the pre-incubation mixture so that the final concentration became 0.1 nM and the mixture was incubated overnight at 4°C. Both of the bound and non-bound (free drug is precipitated by sticking with DCC)  $1\alpha,25-(\text{OH})_2[^3\text{H}]\text{VD}_3$  in the mixture was centrifuged after treatment of dextran coated charcoal, liquid scintillation cocktail (ACS-II) was added to the supernatant, and the radioactivity of the resultant mixture was measured.

The binding affinity of a compound to be tested for the Vitamin D receptor was expressed by a relative intensity ratio based on 100 for  $1\alpha,25-(\text{OH})_2[^3\text{H}]\text{VD}_3$  by determining the concentration which inhibits the binding of the hot by 50%.

c.f. {20epi  $1\alpha,25(\text{OH})_2\text{VD}_3$  の VDR への結合活性}

- chicken intestine VDR 120
- bovine thymus VDR 500

Binding affinity of 20-epi  $1\alpha,25-(\text{OH})_2\text{VD}_3$  to VDR

Exhibit 2  
P. 2  
(Translation)

7 → 8 (#323)

側鎖部 sulfone 980 mg (3 eq) in dry THF (1.5 ml) を Ar 雰囲気下, HMPA 1.5 ml (7 eq) を加え一様とした後,  $-78^{\circ}\text{C}$  に冷却した。n-BuLi (1.6 M in n-hexane) 2.3 ml (3 eq) を滴下し  $-78^{\circ}\text{C}$  で 20 min かくはん後 ヨード体 7 525 mg (1.20 mmol) in dry THF (2 + 洗い込み 1 ml) を滴下。  $-78^{\circ}\text{C}$  で 1 hr かくはん後 反応液に sat  $\text{NH}_4\text{Cl}$  を加えて EA 抽出。有機層をあわせて brine で洗い。  $\text{MgSO}_4$  上脱水。ろか。エバポレート。シリカゲルカラム (EA:n-hex=1:8) にて精製し無色油 8 503 mg (y. 72%) を得ると共に 145 mg の原料 7 を回収 (28%)

Side chain sulfone 980 mg (3 eq) in dry THF (1.5 ml) was added to HMPA 1.5 ml (7 eq) under Ar atmosphere and the mixture was cooled to  $-78^{\circ}\text{C}$  after make the mixture homogeneous. n-BuLi (1.6 M in n-hexane) 2.3 ml (3 eq) was added dropwise to the mixture and stirred for 20 min at  $-78^{\circ}\text{C}$ . Iodo form 7 525 mg (1.20 mmol) in dry THF (2 + rinse 1 ml) was dropwise added to the mixture and stirred for 1 hr at  $-78^{\circ}\text{C}$ . Sat.  $\text{NH}_4\text{Cl}$  was added to the mixture and the resultant mixture was extracted with EA. The extract was combined with organic phase and this solution was washed with brine, dried over  $\text{MgSO}_4$ , filtrated, and evaporated. The residue was purified by silica gel column chromatography (EA:n-hex = 1:8), 503 mg (y. 72%) of colorless oil 8 was obtained with 145 mg of the starting material 7 (28%) was recovered.

8 → 9 (#310)

8 165 mg (0.28 mmol) を dry THF 3 ml, dry MeOH 3 ml にとり  $\text{Na}_2\text{HPO}_4$  3.0 g, 5% Na-Hg 9.8 g を加えて Ar 下 rt でかくはん overnight. 反応液を ether で希釈しセライトろか。有機層を brine で洗い。  $\text{MgSO}_4$  上脱水。ろか。エバポレート。シリカゲルカラム (EA:n-hex=1:9) にて精製し 9 無色油 80 mg (y. 64%) を得ると共に原料 11 mg (7%) を回収。

8 165 mg (0.28 mmol) was dissolved in dry THF 3 ml and dry MeOH 3 ml,  $\text{Na}_2\text{HPO}_4$  3.0 g, 5% Na-Hg 9.8 g was added to the mixture and stirred overnight under Ar atmosphere at rt. The reaction mixture was diluted with ether and the resultant mixture was filtered through celite. The filtrate organic phase was washed with brine, dried over  $\text{MgSO}_4$ , filtrated, and evaporated. The residue was purified by silica gel column chromatography (EA:n-hex = 1:9), 80 (y. 64%) mg of colorless oil 9 was obtained with 11 mg (7%) of the starting material was recovered.

9 → 10 (#316)

ホコ体 9 80mg (0.18 mmol) を MeOH 3 ml に溶解し、TsOH·H<sub>2</sub>O 174 mg (0.91 mmol) を加えて rt かくはん overnight. 反応液から MeOH をエバポレートし、シリカゲルカラム (EA:n-hex = 1:2) にて精製. 無色 oil 43 mg (y. 85%) を得る.

The protected form 9 80 mg (0.18 mmol) was dissolved in MeOH 3 ml, TsCl·H<sub>2</sub>O 174 mg (0.91 mmol) was added to the mixture and stirred overnight at rt. MeOH was evaporated from the reaction mixture and the residue was purified by silica gel column chromatography (EA:n-hex = 1:2), 43 mg (y. 85%) of colorless oil was obtained.

10 → 11 (#326)

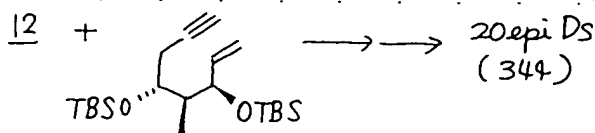
アルコール 10 117mg (0.41 mmol), dry CH<sub>2</sub>Cl<sub>2</sub> (10 ml), 4ÅMS 30 mg を Ar 下 rt で 5 分間かくはんする. TPAP 84 mg (0.24 mmol) を加えて 1 hr 20 min 後 反応液を small pad of silica gel 上 3 かし. エバポレート. シリカゲルカラム (EA:n-hex = 1:1) にて精製. 100 mg (y. 87%) を得る.

The alcohol 10 117 mg (0.41 mmol) was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (10 ml), 4ÅMS 30 mg was added to the mixture and stirred for 5 min under Ar atmosphere at rt. TPAP 84 mg (0.24 mmol) was added to the mixture and the resultant mixture was filtered through small pad of silica gel after 1 hr 20 min. The filtrate was evaporated and the residue was purified by silica gel column chromatography (EA:n-hex = 1:1), 100 mg (y. 87%) was obtained.

11 → 12 (#334)

(bromomethyl)triphenyl phosphonium bromide 389 mg (5 eq) in dry THF (1.5 ml) を Ar 下 -60°C に冷却し 1.0 M NaHMDS 0.86 ml (4.8 eq) を加え -60°C で 1 hr 反応させた後. 11 50 mg (0.18 mmol) in dry THF (1.5 ml) に transfer する. -60°C → 0°C → rt へと昇温し 1 hr 反応させる. 反応液に n-ヘキサンを加え セライト 3 かし 3 液をエバポレートして シリカゲルカラム (EA:n-hex = 1:8 → 1:3) にて精製. 12 36 mg (y. 56%) の淡黄油を得る.

(Bromomethyl)triphenyl phosphonium bromide 389 mg (5 eq) in dry THF (1.5 ml) was cooled to -60°C under Ar atmosphere and 1.0 M NaHMDS 0.86 ml (4.8 eq) was added to the mixture. The resultant mixture was reacted for 1 hr at -60°C and the mixture was transferred to 11 50 mg (0.18 mmol) in dry THF (1.5 ml). The reaction mixture was reacted for 1 hr under the reaction temperature was warmed -60°C → 0°C → rt. n-Hexane was added to the reaction mixture and filtered through celite. The filtrate was evaporated and the residue was purified by silica gel column chromatography (EA:n-hex = 1:8 → 1:3), 36 mg (y. 56%) of pale yellow oil 12 was obtained.



12 17mg (0.048mmol) を toluene 0.3ml に溶かし Et<sub>3</sub>N 0.45ml を加える。(Ar) (dba)<sub>3</sub>Pd<sub>2</sub>·CHCl<sub>3</sub> 1.9mg (0.03eq), Ph<sub>3</sub>P 2.5mg (0.3eq) を加え rt でかくはんしつ A 環部 13mg (0.034mmol) in toluene (150μl + 50μl) を加える。赤黒い溶液を rt で 10min かくはんすると黄色溶液となる。120℃の oil bath 上 2.5hr 反応させる。反応液をろか。ショートカラム (SiO<sub>2</sub>, EA:n-hex = 1:3) に付し黄色油を得る。(精製せず=次の反応へ。)

ホブ体を MeOH 1ml にとかし CSA 11mg (0.047mmol) を加えて Ar 下 rt で overnight かくはん。MeOH を溜まし水を加え EA 抽出。有機層をあつめ brine で洗う。MgSO<sub>4</sub> 上脱水ろかエバポレート。シリカゲルカラム (EA:n-hex = 1:1) にて精製。無色結晶 9.3mg (y. 63%) を得る。

#### <HPLCによる精製>

カラム: LiChrosorb RP-18 (7μm), 10×250, No. 301291

溶媒: Acetonitrile : 水 = 70 : 30

recycler をつけて 流速 7.0 ml/min

12 17 mg (0.048 mmol) was dissolved in toluene 0.3 ml, Et<sub>3</sub>N 0.45 ml was added to the mixture (under Ar atmosphere). (dba)<sub>3</sub>Pd<sub>2</sub>·CHCl<sub>3</sub> 1.9 mg (0.03 eq), Ph<sub>3</sub>P 2.5 mg (0.3 eq) were added to the mixture. A-ring part 13 mg (0.034 mmol) in toluene (150 μl + 50 μl) was added to the mixture under stirring of the mixture at rt. The resultant red-black colored solution was changed to yellow solution during stirring for 10 min at rt. The resultant mixture was reacted for 2.5 hr in an oil bath at 120 °C. The reaction mixture was filtered, the filtrate was evaporated, and the residue was treated with short column chromatography (SiO<sub>2</sub>, EA:n-hex = 1:3), yellow oil was obtained. (The next reaction was carried out without purification)

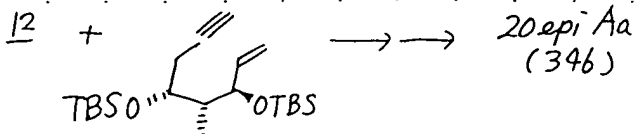
The protected form was dissolved in MeOH 1.0 ml, CSA 11 mg (0.047 mmol) was added to the mixture, and stirred overnight at rt under Ar atmosphere. MeOH was evaporated, water was added to the resultant residue and extracted with EA. The combined organic phase was washed with brine, dried over MgSO<sub>4</sub>, filtrated, and evaporated. The residue was purified by silica gel column chromatography (EA:n-hex = 1:1), 9.3 mg (y. 63%) of colorless crystal was obtained.

#### <Purification by HPLC>

column: LiChrosorb RP-18 (7 μm), 10 x 250, No. 301291

solvent: Acetonitrile : water = 70 : 30

flow rate 7.0 ml / min with recycler



12 15 mg (0.042 mmol) を toluene 0.3 ml に溶かし Et<sub>3</sub>N 0.45 ml を加える (ArF)  
(dba)<sub>3</sub>Pd<sub>2</sub>·CHCl<sub>3</sub> 1.7 mg, Ph<sub>3</sub>P 2.5 mg を加え rt で 10 min かくはんし A 環部  
13 mg (0.034 mmol) in toluene (150 μl + 50 μl) を加え 10 min かくはん. 120 °C の  
oil bath 上 4 hr 反応させる. 反応液をセライトでろし. ショートカラム (EA:n-hex  
= 1:3, SiO<sub>2</sub>) に付し. 黄色油を得る.

ホゴ体と MeOH 1 ml にとり CSA 11 mg (0.047 mmol) を加えて ArF rt で  
overnight かくはん. MeOH を溜去し. 水を加え EA 抽出. 有機層を brine で  
洗い MgSO<sub>4</sub> 上脱水ろす. エバポレート. シリカゲルカラムにて (EA:n-hex = 1:1)  
精製後 無色結晶 4.5 mg (y 31%) を得る.

<HPLCによる精製>

20 *epi* Ds と同様の条件

12 15 mg (0.042 mmol) was dissolved in toluene 0.3 ml, Et<sub>3</sub>N 0.45 ml was added to the mixture (under Ar atmosphere). (dba)<sub>3</sub>Pd<sub>2</sub>·CHCl<sub>3</sub> 1.7 mg, Ph<sub>3</sub>P 2.5 mg were added to the mixture. A-ring part 13 mg (0.034 mmol) in toluene (150 μl + 50 μl) was added to the mixture under stirring at rt and the mixture was stirred for 10 min. The resultant mixture was reacted for 4 hr in an oil bath at 120 °C. The reaction mixture was filtered through celite, the filtrate was evaporated and the residue was treated with short column chromatography (SiO<sub>2</sub>, EA:n-hex = 1:3), yellow oil was obtained.

The protected form was dissolved in MeOH 1.0 ml, CSA 11 mg (0.047 mmol) was added to the mixture, and stirred overnight at rt under Ar atmosphere. MeOH was evaporated, water was added to the resultant residue and extracted with EA. The combined organic phase was washed with brine, dried over MgSO<sub>4</sub>, filtrated, and evaporated. The residue was purified by silica gel column chromatography (EA:n-hex = 1:1), 4.5 mg (y. 31%) of colorless crystal was obtained.

<Purification by HPLC>

same condition as 20 *epi* Ds.